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Insensitive Acetylcholinesterase Causes Resistance to Organophosphates in Australian *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)

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Abstract: Organophosphates are valuable insecticides used to control *Helicoverpa armigera* on cotton in Australia. Those most commonly used for *Helicoverpa* spp. control are profenofos, parathion-methyl and chlorpyrifos. However, there is an emerging organophosphate-resistance threat in Australian *H. armigera*, which is compounded by cross-resistance between profenofos and parathion-methyl. An insensitive acetylcholinesterase has been identified as the common resistance mechanism. No resistance to chlorpyrifos has been detected and acetylcholinesterase remains fully sensitive to the chlorpyrifos oxon.

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Key words: organophosphate; insensitive acetylcholinesterase; resistance; *Helicoverpa armigera*

Insecticide resistance in the cotton bollworm *Helicoverpa armigera* Hübn. is a continuing threat to the economic production of cotton in Australia.[1–6] Chemical insecticides are currently essential for the control of *H. armigera* on cotton and are likely to remain an important component of control strategies for the foreseeable future. The development of resistance had been delayed by an insecticide resistance management strategy for *H. armigera*, but levels of resistance have gradually increased.[1,6–8] Organophosphates have long been used for *H. armigera* control, and are effective larvicides, but they were not used routinely on cotton since more cost-effective insecticides were available. However, as resistance to pyrethroids, endulsulfan and carbamates has increased, so has the use of alternative chemicals such as the organophosphates, particularly late in the cotton growing season.

While resistance to profenofos was detectable from time to time by bioassay, organophosphate resistance did not give control problems in the field.[4] More recently, however, increasing resistance of *H. armigera* to alternative control chemicals such as endosulfan, pyrethroids and carbamates has resulted in an expanded use of the organophosphates (parathion-methyl, profenfos and chlorpyrifos) on cotton and other crops and, as a consequence, there is an emerging and serious field resistance problem in Australia.[9] This situation has been compounded by bioassay studies which indicate cross-resistance between profenofos and parathion-methyl in *H. armigera*. Selection of field-collected *H. armigera* by profenofos resulted in a high level of resistance in third-instar larvae, to both profenofos (92-fold) and parathion-methyl (52-fold). *H. armigera* larvae remain susceptible to chlorpyrifos.

Our biochemical studies have identified an insensitive acetylcholinesterase (AChE) as the resistance mechanism causing resistance to parathion-methyl, and which presumably causes cross-resistance between that compound and profenofos in Australian *H. armigera*. AChE from the profenofos-resistant *H. armigera* was approximately eight times less sensitive to inhibition by paraoxon-methyl and was also less sensitive to inhibition by profenofos, than AChE from susceptible *H. armigera*. Acetylcholinesterase remains fully sensitive to chlorpyrifos. It is not yet clear how this is associated with insensitive AChE responsible for methomyl and thiodicarb resistance, recently reported in this species.[3]

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Factors Affecting Foliar Retention of Some Model Adjuvant Oil-in-Water Emulsions

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Abstract: The foliar retention of dilute oil-in-water emulsions containing three chemical types of adjuvant oil formulated either as ECs or EWs was compared in track-sprayer experiments using the water-soluble tracer, fluorescein. Although both types of emulsion enhanced tracer deposition onto water-repellant pea and barley foliage, compared with sprays containing no adjuvant, consistently better retention was achieved using the EW emulsions, irrespective of oil composition. On young plants, fluorescein retention using the EC emulsifier alone was similar to that from the corresponding emulsions. However, on older plants, retention from EC emulsions was superior to that from the EC emulsifier. Comparable effects were not observed using the EW stabiliser, which had little influence on the efficiency of spray deposition. The retention behaviour of the various oil-based adjuvants is discussed in relation to their effects on spray quality and their formulation ingredients.

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Key words: adjuvant oils; oil-in-water emulsions; spray retention; spray quality

1 Introduction

It is well known that oil-based adjuvants can increase the biological activity of many foliage-applied herbicides and of some fungicide formulations. Although this beneficial effect is generally attributed to increased pesticide uptake,[1] the precise mechanisms of action of these adjuvant oil-in-water emulsions remain poorly understood. In particular, their influence on the efficiency of spray delivery has been largely overlooked.

In the present work, we have measured the foliar retention of oil-in-water emulsions containing three chemical types of adjuvant oil, prepared either as emulsifiable concentrates (ECs), using a surfactant emulsifier, or as concentrated oil-in-water emulsions (EWs) formulated with a polymeric stabiliser. Retention performance was also examined in relation to spray quality data obtained using a phase-Doppler particle analyser (PDPA).

2 Methods

Peas (*Pisum sativum* L. cv. Meteor; three plants per pot) and barley (*Hordeum vulgare* L. cv. Triumph; 10 plants per pot) were raised from seed in pots of compost and maintained in a controlled environment room. Spray applications were made 18 or 22 days (pea) and 10 or 15 days (barley) after sowing. Three candidate oils, Light Liquid Paraffin B.P. (LLP) (mineral oil ex Thornton Ross, UK), soya oil (S) (refined vegetable oil ex Seatons, UK) and methyl soyate (MS) (fatty acid methyl ester, Edenor ME Sj, ex Henkel, Germany) were prepared as ECs with a surfactant blend of Ethylans D253 + C12AH (Akcros Chemicals UK; 1 + 1 by weight; 100 g litre^{-1}) or as EWs with a polymeric stabiliser (Mowiol 3-83 ex Clariant, Germany; 24 g litre^{-1}). Diluted ECs and EWs containing 5–20 g oil litre^{-1}, corresponding rates of emulsifier or stabiliser alone, a nonylphenol surfactant (NP10EO, Agral, ex Zeneca, UK) and a solvent-based bench-mark (acetone + water 1 + 1 by volume), were applied to target foliage at *c.* 200 litre ha^{-1} using a laboratory track-sprayer fitted with an even-spray nozzle. Sodium fluorescein (0·05 g litre^{-1}) was included in all spray solutions; tracer recoveries from sprayed foliage were quantified by spectrofluorimetry and expressed as deposits per unit emulsion (DUE) values, *viz.* ng fluorescein per g dry weight foliage per g fluorescein applied per ha.

3 Results and discussion

Addition of oil ECs and EWs to aqueous sprays enhanced fluorescein deposition on water-repellent pea

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